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Growth of Containerized Loblolly Pine with Specific Ectomycorrhizae after 2 Years on an Amended Borrow Pit

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A borrow pit with exposed subsoil in South Carolina was graded level and deep subsoiled. Plots were amended with processed sewage sludge or commercial fertilizer followed by a seeding with fescue. Container-grown loblolly pines colonized with *Pisolithus tinctorius*, *Thelephora terrestris*, or no ectomycorrhizae were planted by hand on the plots one year after site preparation.

Two years after planting on sludge-amended plots, seedlings initially colonized with *Pisolithus* had greater height, root-collar diameter, and seedling volume (D^2H) than *Thelephora* or control seedlings. The means for these three growth parameters on seedlings planted on fertilized plots were no different between *Pisolithus* and *Thelephora* seedlings, but *Pisolithus* seedlings were greater than controls. There was no difference in survival among mycorrhizal treatments on the sludge plots. Survival and seedling volume were integrated into plot volume indices (PVI). Seedlings on sludge plots had greater PVI than seedlings on fertilized plots. *Pisolithus* seedlings on sludge plots had 265 and 528% greater PVI after two years than *Thelephora* or control seedlings.

Containerized loblolly seedlings tailored with *Pisolithus* can be successfully established and rapid growth obtained on a subsoiled borrow pit amended with sewage sludge. This procedure may be applicable to thousands of acres of similar borrow pits left by highway and construction work.

INTRODUCTION

ECTOMYCORRHIZAL fungi are ubiquitous in disturbed soils of natural forests. Trees in these forests are usually colonized by symbionts ecologically adapted to the existing physical, chemical, and biological soil conditions (Mikola, 1973). Consequently, pine seedlings in recently harvested forests rarely suffer from ectomycorrhizal deficiencies. The afforestation of grasslands or adverse sites with pines, however, is usually met with failure unless the seedlings are adequately colonized by ectomycorrhizal fungi at planting time. Even nursery seedlings well colonized with symbionts often fail to survive and grow on severely disturbed sites if the fungal symbionts on the seedlings roots are not adapted to the soil conditions on the site (Marx, 1977).

The cultural procedures used to produce pines in nurseries favor symbiotic fungi adapted to high soil moisture and fertility. *Thelephora terrestris* (Tt), one of the most common symbionts in southern nurseries, is an example. Unfortunately, the inability of this symbiont to function on harsh sites results in poor initial host survival and growth. *Pisolithus tinctorius* (Pt) is ecologically adapted to strip-mined and other disturbed sites (Schramm, 1966), and by tailoring pine seedlings in the nursery with this symbiont, tree survival and growth is improved on disturbed lands (Marx and Artman, 1979).

Borrow pits are surface-mined sites used to supply fill soil for building construction, dams, and highways. These pits, created by removing several meters of upper soil layers, often become

severely eroded and leave a gullied landscape without vegetative cover along highways and around cities. The exposed and eroded subsoil in these pits is usually compacted, droughty, stony, and inherently low in fertility. From personal observations of several early reforestation attempts, poor survival and growth were noted where standard planting techniques were used to establish native pines on borrow pits in the piedmont region of the southern United States. New techniques of site amelioration followed by planting pines with specific ectomycorrhizae tolerant to adverse soil conditions might be combined in attempts to stabilize these sites. Deep subsoiling to fracture the compacted soil may be a useful technique (Berry, 1977). Natural forests accumulate, store, and recycle nutrients from organic matter deposited on the soil by the existing plant cover. On surface-mined areas, where these organic horizons have been removed, sewage sludge has been successfully added to initiate nutrient cycling (Smith and Evans, 1977). Pine trees with ectomycorrhizae ecologically adapted to adverse soil conditions can also be useful (Marx, 1977). These cultural practices used together in the proper sequence might succeed in correcting problems accounting for past afforestation failures on borrow pits. The use of container-grown pine seedlings instead of nursery produced bare-root seedlings may also have merit for establishing pines on borrow pits (Ruehle and Marx, 1977). The root plug developed in containers can be transplanted without any loss of ectomycorrhizae, a problem often encountered during the lifting of bare-root nursery seedlings. Containerized seedling production can also be scheduled to provide plantable size seedlings for the season with optimum rainfall and temperature.

The aim of this study was to use a combination of soil amelioration techniques and ectomycorrhizae to determine their effects on the establishment of loblolly pine (*Pinus taeda* L.) on a typical borrow pit in the piedmont of South Carolina.

METHODS AND MATERIALS

A borrow pit site was selected on the Savannah River Plant located near Aiken, South Carolina. This site was originally sandhill soil of the Eustis series with surface layers of gray sand and subsoils of very fine, compact, yellowish-red sandy clay. This pit was created during 1950–1952 by the removal of 2 to 6 meters of upper soil layers. The exposed stratum was a heavy, impervious clay characterized by extremes in particle-size distribution and low nutrient content. Loblolly pine seedlings were planted on this pit in 1953 following standard procedures. After 22 years' growth, the trees were only 5 to 15 cm in root-collar diameter and 2.5 to 5 m tall.

In June 1975, all trees were removed and the site was leveled by grading. Two months later the site was subsoiled to a depth of 1 m on 1.2 m centers in both north-south and east-west directions. The dry conditions at this time resulted in excellent fracturing of the compacted soil. The site was then double disked to break clods and smooth ridges created by the subsoiler.

In September, 30 plots each 7.3 × 7.3 m were arranged in two rows across the site with a 6-m buffer zone separating all plots. Processed sewage sludge from Athens, Georgia, was broadcast over the soil in 15 plots at the rate of 0.9 m^3/plot (approximately 1.3 cm deep). The remaining 15 plots, referred to hereafter as fertilizer plots, received broadcast applications of 560 kg/ha of commercial 10-10-10 fertilizer and 2,240 kg/ha of dolomitic limestone. All plots were double disked 10 to 15 cm deep to incorporate the amendments. The perimeter of each plot was disked to form a ridge to prevent loss of sludge and fertilizer. Three weeks later the entire study area was sown with fescue [*Festuca arundinacea* Schreb. (Ky 31)] at a rate of 30 kg/ha to control erosion and retard nutrient loss until the pine seedlings were planted the following year.

Containerized loblolly pine seedlings were produced in a greenhouse in Athens, Georgia. A growing medium composed of a peatmoss-vermiculite mixture (1:1 v/v) was mixed with pulverized 10-10-10 commercial fertilizer (9 g/100 *l* of growing medium). Inocula of Pt (isolate 138) and Tt (isolate 201) were produced in peatmoss-vermiculite-nutrient medium and processed as reported earlier (Marx and Bryan, 1975). Inoculum was mixed in a 1:6 v/v ratio with the growing medium. Autoclaved inoculum of Pt was mixed with growing medium at the same rate for the control seedlings. The two groups of Styroblock 8® containers* filled with medium and viable inoculum were placed in the greenhouse and the control group of containers with medium and sterile inoculum were placed in a filtered-air growth room (Marx and Bryan, 1969) to maintain seedlings in a nonmycorrhizal condition. In July 1976, all units were seeded with several seeds per cavity and, after seed germination, seedlings

*The use of trade names in this publication is for the information and convenience of the reader, and does not constitute an official endorsement by the U.S. Department of Agriculture or the Forest Service.

*Table 1. Survival and Growth of Containerized Loblolly Pine Seedlings after Two Years on a Borrow Pit in South Carolina**

Amendment	Mycorrhizal condition	Survival %	Height cm	Root-collar diameter cm	Seedling volume† cm^3
Sludge	*Pisolithus* (Pt)	91.2 a‡	107.2 a	3.0 a	1215.4 a
	Thelephora (Tt)	73.6 a	76.0 b	1.9 b	390.6 b
	Control	68.0 a	70.7 b	1.6 b	236.5 b
	$\bar{X}$	77.6	81.1	2.2	614.2
Fertilizer	*Pisolithus* (Pt)	96.0 a	34.5 a	0.9 a	38.0 a
	Thelephora (Tt)	88.0 b	31.4 ab	0.9 ab	35.0 ab
	Control	89.6 b	26.3 b	0.7 b	16.0 b
	$\bar{X}$	91.2	30.7**	0.8**	29.7**

*Means of survivors from 25 test seedlings initially planted in each of five plots. Each number followed by a common letter within groups of parameters is not significantly different at the $p=0.05$ confidence level.

†Seedling volume (cm^3) = (root-collar diameter)^2 × height.

**Denotes significant differences ($p=0.01$) between groups according to Student's *t*-test.

were thinned to one per cavity. All seedlings were watered as needed with tap water. No additional fertilizer was added. Additional seedlings were grown in the greenhouse in styroblocks containing only growing medium to provide seedlings for the border rows.

In November 1977, ten randomly selected seedlings per treatment replicate were measured and their roots were visually examined for ectomycorrhizae. Inoculated seedlings, both Pt and Tt, averaged 14.7 cm in height and 2.1 mm in root-collar diameter. Control seedlings averaged 13.1 cm in height and 1.9 mm in root-collar diameter. Seedlings were hand planted in each plot in five rows of five seedlings each on 1.2 m centers. A border row was planted around each plot. The six treatments, replicated five times, were assigned to plots at random.

Biomass samples were collected from the ground cover on each plot at planting time and after each of the next two growing seasons. Each plot of 25 test seedlings created a grid containing 16 squares measuring 1.2 m^2. Three squares were selected at random at each sampling period and a 0.3-m^2 frame was placed in the center of each. All grass within each frame was clipped by hand to within 1 cm of the ground. All clippings from each plot were combined in a tared paper bag, ovendried at 90°C for 72 h, and weighed.

In November 1978, trees in all plots were measured for root-collar diameter and height. Five randomly selected trees per replicate were dug from plots within each treatment to visually assess ectomycorrhizal development. Soil samples were removed to 10 cm depth from each plot, air dried at room temperature for 10 days, and processed for nutrients by the 0.05N HCl + 0.02N H_2SO_4 extraction and soil organic matter was determined by the Walkely-Black wet oxidation method (Wells et al., 1973). Foliar samples of current-year needle bundles were removed from five randomly selected trees per plot, combined into one sample, and dried at 90°C for 72 h prior to tissue analysis for total N by Kjeldahl and for the other elements by dry ash methods (Wells et al., 1973). Concentrations of phosphorus were determined colorimetrically and all other ions were assayed by atomic absorption. Analyses of foliar and soil samples were performed by Carol G. Wells, U.S. Department of Agriculture, Forest Service, Forestry Services Laboratory, Research Triangle Park, North Carolina.

Analyses of variance were made on all data following procedures for a completely random design, and treatment differences were evaluated with Duncan's Multiple Range Test (P = 0.05) and combined group means were tested with Student's t-test (P = 0.01).

RESULTS

Seedlings with Pt ectomycorrhizae at planting had significantly better survival at the end of the study than seedlings colonized with Tt or nonmycorrhizal seedlings in the fertilized plots (Table 1). Survival of seedlings on sludge-amended plots was not significantly affected by ectomycorrhizal treatment. Dense grass and deer bedding caused considerable mortality of pine seedlings in sludge plots, with damage by deer causing greater within-treatment variation in seedling survival than in the fertilizer plots.

In sludge plots, seedlings with Pt ectomycor-

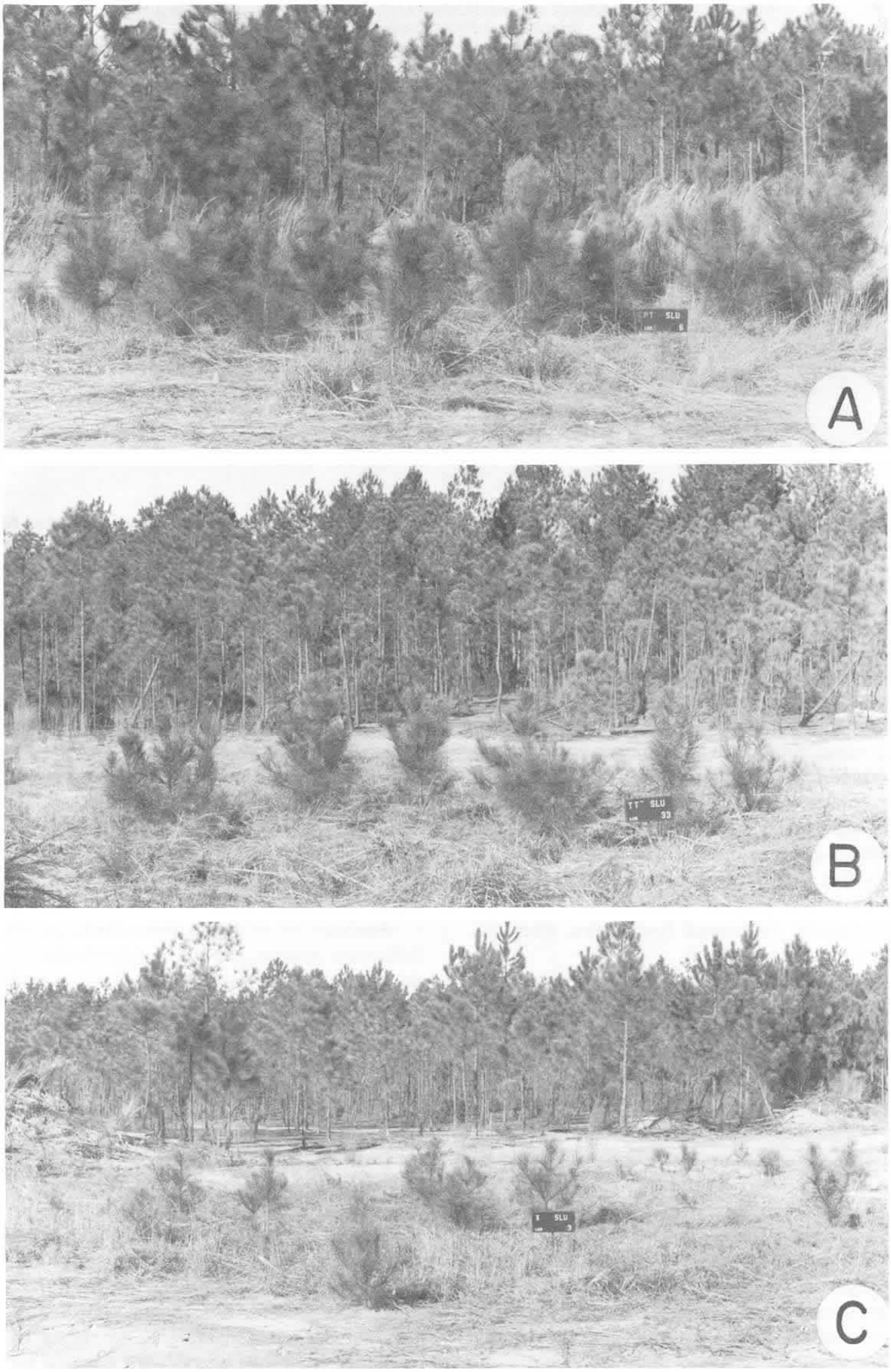

Figure 1. Two-year growth of containerized loblolly pine seedlings colonized with specific ectomycorrhizal fungi and planted in borrow pit plots amended with sewage sludge. A—Pisolithus tinctorius, *B*—Thelephora terrestris, *C—control (nonmycorrhizal).*

rhizae at planting had significantly greater height, root-collar diameter, and seedling volume than seedlings with Tt ectomycorrhizae or with none (Table 1, Fig. 1). The average volume of seedlings with Pt ectomycorrhizae was over 200% greater than seedlings with Tt and over 400% greater than seedlings initially without ectomycorrhizae. In the fertilizer plots, seedlings with Pt ectomycorrhizae were not significantly larger than seedlings with Tt ectomycorrhizae, but they were significantly greater in height, root-collar diameter, and seedling volume than seedlings lacking ectomycorrhizae at planting.

Survival and growth data were integrated into plot volume indices (PVI) (Marx et al., 1977) and the same growth differences due to treatments were noted (Fig. 2). In the sludge plots, seedlings with ectomycorrhizae at planting had PVI that were 265 and 528% greater than seedlings with Tt ectomycorrhizae or no mycorrhizae. In the fertilizer plots, PVI of seedlings with Pt were no different from those with Tt, but they were significantly greater by 158% than those of nonmycorrhizal seedlings. As a group, seedlings on sludge plots had 900% greater PVI than those on fertilizer plots.

Chemical analyses of soil showed that major elements and organic matter were low in the fertilizer plots by the end of the study (Table 2). There was significantly more organic matter, N, and P, and less Mg in sludge-amended plots than in fertilizer plots. Sludge plots average 1 pH unit lower than fertilized and limed plots. Seedlings with different ectomycorrhizal treatments did not affect the chemical status of the soil. Needles from seedlings in sludge plots had more N, Mn, Na, and Zn than those from seedlings in fertilizer plots (Table 3). However, needles from seedlings in fertilizer plots had more Al, Ca, Fe, and Mg than needles from seedlings in sludge plots. Although there were differences in metal ion concentrations in the foliage of seedlings from sludge amended plots versus the fertilized plots, there were no symptoms of metal toxicity. Needle concentrations of P, K, and Cu were not affected by soil amendments. In the sludge plots, seedlings with Pt ectomycorrhizae at planting had less foliar N at the end of the study than those with Tt ectomycorrhizae or with none. Ectomycorrhizal condition of seedlings failed to affect the foliar concentrations of other elements assayed.

Root evaluations of ectomycorrhizae on seedlings after two years revealed that Pt was dominant on seedlings in all plots planted with Pt seedlings, and also accounted for 20% of all the ectomycorrhizae on seedlings initially with Tt ectomycorrhizae and about 10% of the ectomycorrhizae on seedlings initially without ectomycorrhizae. Tt constituted about 70% of all the ectomycorrhizae on seedlings with Tt at planting and nearly 50% of the ectomycorrhizae on those without ectomycorrhizae at planting. An unidentified white symbiont accounted for nearly 25% of the ectomycorrhizae on seedlings in all plots.

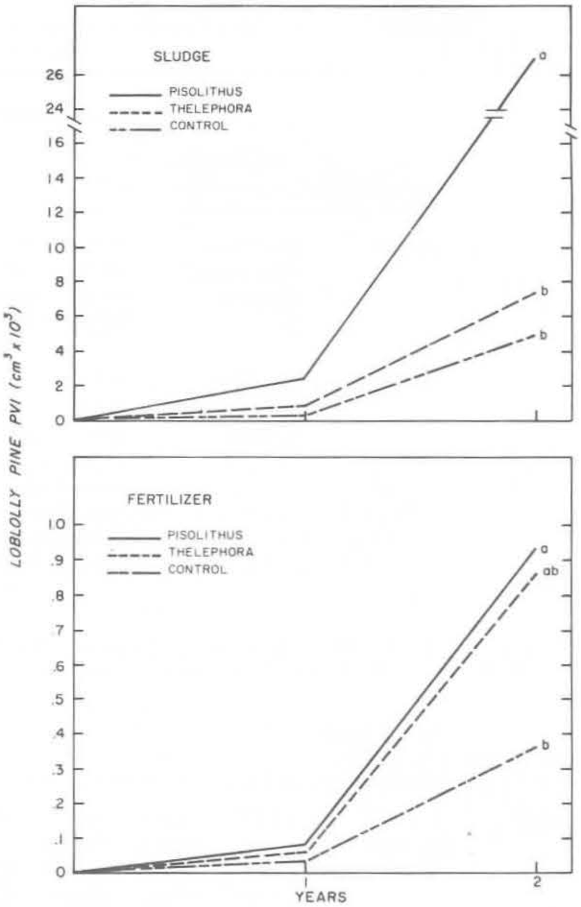

Figure 2. Plot volume indices (PVI) of loblolly pine seedlings with Pisolithus, Thelephora, *or no ectomycorrhizae at planting after two years in amended plots on a borrow pit in South Carolina. PVI = (root-collar diameter)² × height × number of surviving seedlings per plot. Points within each fertility treatment at the two-year line followed by the same letter are not significantly different (P = 0.05). Note the difference in the scale between sludge and fertilizer treatments.*

Ground cover biomass on sludge plots averaged 468 and 510 g/m², respectively, for 1977 and 1978. Biomass on fertilized plots was markedly less than that on sludge plots averaging 69 and 33 g/m² for these two years.

DISCUSSION

Containerized loblolly seedlings can be successfully used to establish trees on a borrow pit. Overall survival on both sludge and fertilized

*Table 2. Chemical Soil Properties on Plots in the Borrow Pit in South Carolina Three Years after Addition of Amendments**

Amendments	Mycorrhizal condition	N (ppm)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Organic matter %	pH
Sludge	*Pisolithus*	523 b†	59 b	7 a	17 a	11 a	1.68 b	4.0 a
	Thelephora	583 b	72 b	9 a	17 a	10 a	1.60 b	3.8 a
	Control	581 b	59 b	10 a	19 a	13 a	1.83 b	3.8 a
	$\bar{X}$	562.5	63.3	8.8	17.7	11.2	1.71	3.84
Fertilizer	*Pisolithus*	109 a	4 a	8 a	13 a	58 b	0.49 a	4.8 b
	Thelephora	128 a	6 a	7 a	13 a	60 b	0.48 a	4.9 b
	Control	103 a	10 a	8 a	16 a	72 b	0.46 a	4.9 b
	$\bar{X}$	127.6‡	6.6‡	7.8‡	14.1‡	63.4‡	0.48‡	4.85‡

*Values are means of five samples, and for the elements, represent extractable fractions.
†Means within columns not followed by the same letter are significantly different ($p=0.05$).
‡Denotes significant differences between group means ($p=0.01$) according to Student's t-test.

*Table 3. Foliar Analysis of Containerized Loblolly Pine Seedlings after Two Years on a Borrow Pit in South Carolina**

Amendment	Mycorrhizal condition	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Mn (ppm)	Fe (ppm)	Na (ppm)	Zn (ppm)	Cu (ppm)	Al (ppm)
Sludge	*Pisolithus*	1.65 b†	0.15 a	0.34 a	0.22 a	0.07 a	256 bcd	40 a	123 a	150 b	5 a	651 a
	Thelephora	1.95 c	0.15 a	0.47 b	0.29 ab	0.10 a	372 cd	49 a	103 a	150 b	4 a	689 a
	Control	1.89 c	0.18 a	0.49 b	0.28 ab	0.10 a	313 d	51 a	103 a	146 b	4 a	802 ab
	$\bar{X}$	1.832	0.162	0.435	0.261	0.091	313.9	46.7	109.8	148.5	3.2	713.8
Fertilizer	*Pisolithus*	1.51 ab	0.13 a	0.42 ab	0.34 b	0.21 b	104 a	106 b	54 a	46 a	3 a	1061 c
	Telephora	1.52 ab	0.14 a	0.34 a	0.32 b	0.19 b	121 ab	146 b	84 a	60 a	4 a	1079 c
	Control	1.49 a	0.14 a	0.43 ab	0.34 b	0.18 b	163 abc	122 b	84 a	61 a	3 a	991 bc
	$\bar{X}$	1.523‡	0.137	0.395	0.334‡	0.193‡	129.4‡	124.9‡	74.1‡	55.8‡	3.2	1043.6‡

*Values are means of five samples.
†Means within columns not followed by the same letter are significantly different ($p=0.05$).
‡Denotes significant differences between group means ($p=0.01$) according to Student's t-test.

plots was as good or better than comparable bare-root seedlings in an adjoining study on this borrow pit (Berry and Marx, 1980). The size of container seedlings was slightly less than comparable bare-root seedlings after two years, i.e., seedling volume on sludge/Pt plots averaged 1,215 cm^3 for container seedlings and 1,885 for bare-root seedlings. The smaller seedling size for container seedlings at planting probably accounted for this smaller volume.

Although the Pt treatment accounted for a marked increase in survival and tree growth on fertilizer plots over the control, even trees with this symbiont were considerably smaller after two years than trees initially without ectomycorrhizae on sludge plots. Although nutrient levels in fertilizer plots were considerably lower at the end of the study than those in sludge plots, they were still not below levels normally encountered in many southern forest soils (Pritchett and Smith, 1970). At favorable temperature and with adequate soil nutrients, root growth of loblolly pine is controlled primarily by soil moisture and aeration (Bilan, 1968). Sludge treatments in this study maintained a moister and better aerated soil than fertilizer treatments. Soil in sludge plots compared to fertilized plots had more cellulose-degrading fungi, slime molds, and larger numbers of yeasts and bacteria.* These observations suggest that processes of mineral recycling are more advanced after three years in borrow pit soil amended with sludge than soil amended with fertilizer. The well-developed grass biomass on sludge plots also shaded the soil to maintain lower surface temperatures and probably allowed better pine root development in the

*Wojcik, V. H. and D. H. Marx. Unpublished data. Southeast. For. Exp. Stn., Forestry Sciences Laboratory, Athens, Georgia.

upper layers during the summer months. Foliar analysis indicated that levels of N, P, and K were satisfactory in the needles of seedlings in both sludge and fertilizer plots (Wells, 1970; Wells et al., 1973). In sludge plots, the lower concentrations of foliar N in *Pisolithus* seedlings was probably a reflection of dilution effect caused by greater top growth compared to *Thelephora* and nonmycorrhizal seedlings. This concentration was still well above amounts found in foliage of loblolly on fertilized forest soils. Therefore, poor physical properties of soil in fertilizer plots probably accounted for poorer root development and less overall growth of seedlings in these plots. The excellent two-year growth of loblolly pine in the sludge plots reflect the improvements to soil fertility and soil moisture provided by sludge amendments.

Grass cover that developed well in sludge plots provided other benefits to borrow pit soil. A portion of the nutrients released from the sludge were taken up by the grass and provided a nutrient reserve in the system. This cover provided adequate soil stabilization for erosion control. Although this borrow pit was graded level and erosion was not a serious problem, if there had been a slope on this site the fertilized plots with little grass cover would have undergone serious erosion.

The poorer growth of seedlings with no mycorrhizae at planting indicates that seedlings with any ectomycorrhizae at planting are better than those with no ectomycorrhizae. Containerization programs should adjust cultural procedures to ensure ectomycorrhizal development on pine seedlings. The improved growth of seedlings with Pt ectomycorrhizae demonstrates the point made by Marx (1977) that certain species of ectomycorrhizal fungi are more beneficial to pines than others, particularly on stressed sites.

Amelioration of any borrow pit physically and chemically unsuited for supporting vegetation probably will require the integration of both cultural and vegetative methods: (1) subsoiling to fracture the indurate soil surface layers, (2) addition of appropriate organic matter to restore necessary physical and biological factors, and (3) a combination of grass cover and forest tree seedlings colonized with beneficial mycorrhizal symbionts ecologically adapted to adverse sites. Such a unified program for stabilizing borrow pits is the key to rapid establishment of a self-perpetuating vegetative cover which can return these nonproductive biological deserts into productive land for trees, wildlife, and water management schemes.

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